Effects of magnesium and zinc deficiencies on growth and protein synthesis in skeletal muscle and the heart

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The effects of magnesium or zinc deficiency on growth, tissue contents of Mg or Zn and protein synthesis have been compared in 4–13-week-old rats. When maintained on Mg-deficient fodder (1·6 mmol/kg) or Zn-deficient fodder (27 μmol/kg) rats showed a reduced weight gain, whereas repletion caused increased growth rates. Pair-feeding experiments showed that this could not be attributed to reduced energy intake only. In rats maintained on Mg-deficient fodder for 14 d [^{3}H]leucine incorporation into skeletal muscle and the heart was reduced by 24–38% compared with pair-fed controls ($P < 0{\cdot}001$–$0{\cdot}002$). The incorporation of [^{3}H]phenylalanine was reduced by 19–31%. Tissue Mg contents, however, were only reduced by 6–7% (not significant). The pair-fed rats showed no reduction in the [^{3}H]leucine incorporation compared with *ad lib.*-fed animals. In rats maintained on Zn-deficient fodder for 15 d [^{3}H]leucine incorporation into skeletal and heart muscle was reduced by 57–64% compared with pair-fed controls. The pair-fed rats showed no reduction in the [^{3}H]leucine incorporation compared with *ad lib.* fed animals. In the Zn-deficient animals the content of Zn was not reduced in the skeletal muscles, whereas there was a small (15%) but significant loss of Zn in the heart. In another experiment, Zn depletion for 17 d caused a reduction in [^{3}H]leucine incorporation of 35–41%. After 5 d of Zn repletion this defect was restored, and the [^{3}H]leucine incorporation was above control level in the skeletal muscles. It is concluded that the intact organism is very sensitive to dietary Mg or Zn deficiency, and that the reduced growth and protein synthesis cannot easily be attributed to the reduction of tissue Mg or Zn content *per se*. This points to the existence of other control mechanisms mediating down-regulation of growth and protein synthesis in response to reduced dietary supplies and the ensuing drop in the plasma concentrations of Mg and Zn.

Growth: Magnesium deficiency: Zinc deficiency: Protein synthesis: Rat

Magnesium and zinc are essential for growth in animals (Schwartz *et al.* 1970; Williams & Mills, 1970; Hunt, 1971) as well as in man (Caddell & Goddard, 1967; Golden, 1988). Nutritional deficiencies of either element lead to a progressive and often marked reduction in weight gain, which is reversible following repletion. This might be related to impaired protein metabolism, and indeed, both Mg and Zn are required for optimum synthesis of proteins in cell-free systems (Schreier & Staehelin, 1973; Hicks & Wallwork, 1987) as well as in intact cells (George & Heaton, 1978; Teresaki & Rubin, 1985). However, when maintained on fodder deficient in Mg or Zn, animals show little or no change in the concentrations of either element in skeletal muscle (Miller, 1969; Hunt, 1971; O'Leary *et al.* 1979; Guigliano & Millward, 1984) or liver (Schwartz *et al.* 1969; Williams & Mills, 1970). Therefore, the impairment of growth and protein synthesis cannot readily be accounted for as the result of reduced availability of Mg or Zn in the major pools of protein-synthesizing cells. Still, several studies have shown that Mg deficiency is associated with reduced net protein synthesis and nitrogen utilization in the intact organism (Menaker & Kleiner, 1952; Schwartz *et al.* 1969), and a recent study demonstrated that in Zn-deficient rats, the in vivo protein synthesis in muscle was inhibited (Giugliano & Millward, 1987).

Taken together, these observations indicate that during nutritional deficiencies of Mg and Zn, the rates of growth and protein synthesis are down-regulated before any major tissue deficiencies have time to develop. Therefore, factors other than changes in the concentrations of Mg and Zn at the sites of protein synthesis must be involved.

The present study was performed to compare the effects of Mg and Zn deficiencies on growth and the in vivo protein synthesis with the tissue contents of these elements. In the following article, the changes in the serum levels of Mg and Zn, insulin-like growth factor-1 (IGF-1), growth hormone and insulin are analysed (Dørup *et al.* 1991).

MATERIALS AND METHODS

Animals and treatment

All experiments were performed using female Wistar rats in the age-range 4–13 weeks. The animals were kept at constant temperature (23 °), humidity (53%) and daylength (12 h). Stainless-steel frames were positioned in the bottom of the cages to prevent the animals from having access to urine or faeces. Until used for experiments, all rats were maintained on a standard fodder containing 50 mmol Mg/kg and 500 μmol Zn/kg (The Altromin Co., Lage, Germany).

Experiments were performed with semi-synthetic Mg-free or Zn-free fodder (EWOS, Södertälje, Sweden). This fodder had an energy content of 14·0 kJ (3·35 kcal)/g and contained (g/kg) 170 protein, 70 fat, 644 carbohydrate, 50 vitamins and minerals, and water content was 66 g/kg.

Mg deficiency was induced by maintaining rats on distilled water and the semi-synthetic Mg-deficient fodder. The mean Mg content of all batches of this fodder was found to be 1·56 (SE 0·07) mmol/kg. The minimum dietary requirement of Mg is considered to be 17 mmol/kg (Warner & Breuer, 1972). Except in the pair-feeding experiment (see below) the control animals were maintained on distilled water and the same fodder supplemented with magnesium chloride to give a final concentration of 50 mmol Mg/kg fodder.

Before the start of the dietary regimen the rats were acclimatized to the semi-synthetic fodder by feeding the Mg-supplemented fodder for 3 d. After this adjustment period the rats were randomly divided into the experimental groups.

Mg repletion was performed after Mg depletion by giving the Mg-depleted animals free access to Mg-enriched fodder (50 mmol Mg/kg).

Pair-feeding was performed by maintaining the control animals on the same amount of fodder, on a body-weight basis, as that consumed by the rats on Mg-deficient fodder during the previous 24 h. The daily food supply was established each morning after assessment (by weighing uneaten fodder) of the food consumed by the Mg-depleted group during the previous 24 h. The pair-fed animals were then each given the same amount of fodder on a body-weight basis, as that consumed in the Mg-depleted group. In addition an *ad lib.*-fed group with free access to fodder was included. All three groups were kept on Mg-free fodder and the Mg-depleted group received distilled water, while pair-fed and *ad lib.*-fed animals were given a $MgCl_2$ solution (40 mmol/l).

Zn deficiency was induced by maintaining 4-week-old rats on distilled water and a semi-synthetic Zn-deficient fodder. The mean Zn content of all batches of this fodder was found to be 27·3 (SE 1·6) μmol/kg, which is well below what is considered to be the minimum dietary requirement of the rat (200 μmol/kg; Warner & Breuer, 1972). The control animals were maintained on distilled water and the same diet supplemented with zinc chloride to a final concentration of 500 μmol/kg fodder (control fodder). The animals were kept in plastic cages with grids constructed of stainless steel. Precautions were taken to minimize trace element contamination.

Before the start of the dietary regimen the rats were acclimatized to the semi-synthetic fodder by feeding the Zn-supplemented fodder for 3 d. After this adjustment period the rats were randomly divided into the experimental groups.

Zn repletion was performed after Zn depletion by giving the Zn-depleted animals free access to Zn-enriched fodder (500 μmol Zn/kg).

In one experiment, the animals were pair-fed by maintaining the controls on the same amount of fodder, on a body-weight basis, as that of the rats on Zn-deficient fodder. A third group was given free access to fodder. All three groups received distilled water. Whereas the Zn-depleted group received Zn-free fodder, pair-fed and *ad lib.*-fed animals were given the same fodder supplemented with $ZnCl_2$ to give a final concentration of 500 μmol/kg fodder.

Incorporation of ^3H activity into protein

The incorporation of ^3H activity from [^3H]leucine or [^3H]phenylalanine into tissue proteins was determined following intravenous injection. The procedure was based on that developed by McNurlan *et al.* (1979) and has previously been described in detail (Dørup & Clausen, 1989). Briefly, under phenobarbital anaesthesia the rats were given an intravenous injection (10 ml/kg body-weight) of a solution containing 154 mmol sodium chloride/l, 100 mmol L-leucine/l and 20 μCi [^3H]leucine/ml or a solution containing 154 mmol NaCl/l, 150 mmol L-phenylalanine/l and 20 μCi [^3H]phenylalanine/ml. After 10 min the animals were decapitated and samples of the tissues indicated excised, frozen and prepared for the isolation and counting of protein as previously described (Dørup & Clausen, 1989).

The incorporation of ^3H activity into total protein was expressed as counts/min (cpm) per g tissue wet weight.

Mg, Zn, and potassium contents and amino acids

For the determination of Mg, Zn and K, samples weighing 15–40 mg were homogenized in 2 ml trichloroacetic acid (TCA; 300 mmol/l) using an Ultra-turrax homogenizer, centrifuged for 10 min at 2770 ***g***, and the clear supernatant fraction taken for further dilution with 2 vol. redistilled water. This dilution was used for determination of the Mg content by atomic absorption (Perkin Elmer 5000, wavelength, 285·2 nm) or the K content by flame photometry (FLM Radiometer with lithium as internal standard). The use of this procedure for Mg and K determination has been described previously in detail (Dørup *et al.* 1988). For Zn determination the clear supernatant fraction was further diluted with 1 vol. redistilled water, and measurements performed using atomic absorption (Perkin Elmer 5000; wavelength 213·9 nm). The recovery of Zn added to TCA extracts of six soleus muscle biopsies was tested in the relevant concentration range. The mean recovery was 103·7 (SE 0·8)%.

In some experiments serum samples were taken from the orbital veins for measurement of amino acid contents. The samples were obtained 3 and 7 min after the injection of labelled amino acid. Amino acid analyses were performed directly on diluted serum using Amino Quant (Hewlett-Packard).

Chemicals and isotopes

All chemicals were of analytical grade. L-[4,5-^3H]leucine (60 Ci/mmol) and L-[4-^3H]phenylalanine (28 Ci/mmol) were obtained from the Amersham International Plc, Amersham, Bucks.

Statistics

All results are given as mean values with their standard errors. The significance of difference was assessed by the two-tailed *t* test for groups of non-paired observations and by one-way analysis of variance. Linear correlation analysis of unweighted values was performed by the method of least squares.

RESULTS

Mg depletion

The effect of Mg deficiency on growth was evaluated in groups of rats which were kept on Mg-deficient fodder for 3 weeks and then repleted by changing to the same fodder supplemented with $MgCl_2$ to a content of 50 mmol/kg. Fig. 1 shows the time-course of changes in body-weight during Mg depletion and subsequent repletion. The onset of growth retardation is much slower than seen during Zn depletion (see p. 498), and a significant reduction in body-weight was not seen until after 7 d. After 17 d the weight gain was 56% of that in the controls and, thereafter, no further growth could be detected. When the animals were repleted they showed an increased relative growth rate compared with the controls, but did not catch up the absolute weight difference which remained almost constant (after 3 weeks of repletion amounting to 37·6 g, compared with a weight difference at the onset of repletion of 41·6 g).

The role of energy and Mg intake was analysed in a pair-feeding experiment. Since the amount of fodder consumed by *ad lib.*-fed animals at a given age is a constant percentage of the actual body-weight, a restriction in food supply to the accurate amount of fodder consumed in the Mg-depleted group would lead to semi-starvation. So in order to investigate the effect of Mg deficiency *per se* and not of combined Mg deficiency–semi-starvation, therefore, we chose to feed the pair-fed animals the same amount on a bodyweight basis, as that consumed by the Mg-deficient group rather than feeding them the same absolute amount of fodder.

As shown in Table 1, the pair-fed rats showed a 33% reduction in weight gain but no significant change in the incorporation of [^{3}H]leucine into muscle protein compared with the *ad lib.*-fed animals. The same experiment showed a further reduction in weight gain (26%) and [^{3}H]leucine incorporation (24–38%) in the Mg-deficient animals compared with the pair-fed controls. These substantial changes in weight gain and [^{3}H]leucine incorporation could not be related to reduced tissue contents of Mg. Thus, following 2 weeks on Mg-deficient fodder, soleus and extensor digitorum longus (EDL) muscles showed a decrease in Mg content of only 6·7% (not significant) and 6·2% (not significant) respectively (pair-fed controls *v.* Mg-depleted). In contrast, serum Mg showed a rapid response to the reduced Mg content of the fodder (see Dørup *et al.* 1991). In the Mg-deficient rats the weight of soleus and EDL muscles showed the same relative decrease as the body-weight (Table 1).

In another experiment, the incorporation of [^{3}H]phenylalanine was evaluated. Table 2 shows that the incorporation of this amino acid into skeletal muscle and the heart ventricle was significantly reduced in animals maintained on Mg-deficient fodder for 17 d. The reduction in soleus, EDL, diaphragm and heart in the Mg-deficient group was 25, 31, 27 and 19% respectively. This should be compared with the values in Table 1 (*ad lib.*-fed *v.* Mg-depleted for 14 d), where the reduction in [^{3}H]leucine incorporation amounted to 35, 35, 32 and 36% in the same tissues respectively.

Measurements of amino acids performed during the [^{3}H]leucine experiments showed no significant differences in the serum concentrations of glycine, isoleucine, lysine, methionine, tyrosine and valine between the Mg-deficient and the control animals. In the Mg-deficient rats, the serum concentrations of threonine and serine were reduced by 25 and 16% respectively.

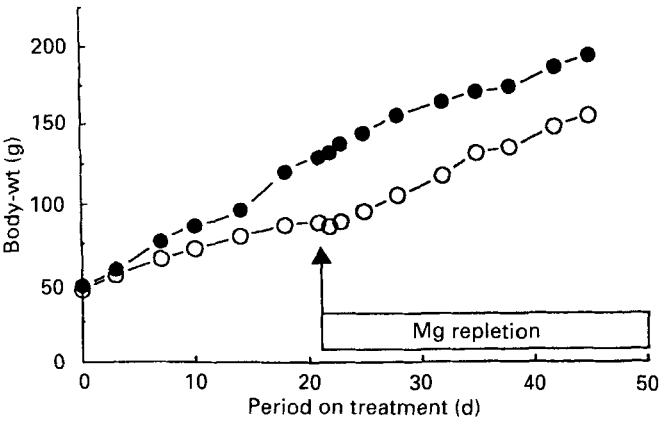

Fig. 1. Effect of magnesium depletion and Mg repletion on body-weight. Groups of 4-week-old rats were maintained on Mg-deficient fodder (1·6 mmol/kg) or on the same fodder supplemented with magnesium chloride to a content of 50 mmol/kg (for details of composition, see p. 494). After 21 d the rats on Mg-deficient fodder were Mg repleted by changing to the Mg-supplemented fodder. Each point represents the mean of five Mg-depleted–repleted (○——○) or six control (●——●) animals. The standard error does not exceed the size of the symbols. The difference between the body-weight of the controls and that of the Mg-depleted–repleted rats was significant ($P < 0·01$ or less) at all points except on days 0 and 3.

Table 1. *Effect of magnesium depletion and pair-feeding on body-weight, muscle weight, Mg content and [^{3}H]leucine incorporation into skeletal muscle and heart ventricle of rats*

(Mean values with their standard errors; no. of animals in parentheses)

Treatment*...	*Ad lib.*-fed controls (*n* 13)		Pair-fed controls (*n* 12)		Statistical significance of difference : *P*	Mg-depleted (*n* 11)		Statistical significance of difference from *ad. lib.*-fed controls: *P*
	Mean	SE	Mean	SE		Mean	SE	
Body-weight (g)								
Day 0	50·0	0·6	50·1	0·6	NS	49·8	0·7	NS
Day 14	103·8	2·5	85·9	2·1	< 0·001	76·2	1·6	< 0·002
Muscle wet wt on day 14 (mg)								
Soleus	37·8	1·1	33·6	1·4	< 0·02	27·1	2·7	< 0·05
EDL	35·7	1·1	32·3	1·3	< 0·05	24·4	0·7	< 0·001
Muscle Mg content on day 14 (μmol/g wet wt)								
Soleus	9·36	0·36	9·49	0·35	NS	8·85	0·37	NS
EDL	10·67	0·29	10·82	0·29	NS	10·15	0·19	NS
Heart ventricle	9·56	0·13	9·48	0·14	NS	9·16	0·15	NS
[^{3}H]leucine incorporation on day 14 (cpm/g wet wt)								
Soleus	13918	573	13448	927	NS	9014	725	< 0·002
EDL	10471	464	10176	675	NS	6787	542	< 0·001
Diaphragm	12118	487	10773	486	NS	8203	575	< 0·002
Heart ventricle	11557	471	11978	566	NS	7414	789	< 0·001

EDL, extensor digitorum longus; NS not significant; cpm, counts/min.
* For details, see pp. 494–495.

Table 2. *Effect of magnesium depletion on [^{3}H]phenylalanine incorporation into skeletal muscle and heart ventricle of rats*

(Mean values with their standard errors; no. of animals in parentheses)

Treatment*...	Controls (*n* 8)		Mg-depleted (*n* 7)		Statistical significance of difference: *P*
	Mean	SE	Mean	SE	
[^{3}H]phenylalanine incorporation (cpm/g wet wt)					
Soleus	4914	252	3698	112	< 0·001
EDL	4731	256	3253	188	< 0·001
Diaphragm	5097	490	3735	204	< 0·05
Heart	4155	302	3369	121	< 0·05

EDL, extensor digitorum longus; cpm, counts/min.

In two control experiments, one using labelled leucine and one using labelled phenylalanine, the serum concentrations of the injected amino acid was measured. From these values the mean serum concentration and specific activity during the experiment could be calculated. After injection of leucine the mean concentration of leucine in the serum of the control animals was 1·27 (SE 0·04) mmol/l (*n* 9) and 1·29 (SE 0·03) mmol/l (*n* 9) in animals Mg-depleted for 16 d (not significant). This value is thirteen times higher than the physiological leucine level in the rat of 0·1 mmol/l (Banos *et al.* 1973). After injection of phenylalanine the mean serum level of this amino acid during the experiment was 1·69 (SE 0·05) (*n* 8) in the control animals and 1·94 (SE 0·10) (*n* 7) in animals Mg-depleted for 17 d ($P < 0·05$). The physiological level of phenylalanine in the rat is 0·05 mmol/l (Banos *et al.* 1973), so the serum concentration had risen 34-fold after injection of the flooding dose. Mean specific activity in both experiments showed no significant difference between controls and Mg-deficient animals. From the calculated mean specific activity in serum during the experiments the incorporation rates could be calculated. In the control group the [^{3}H]leucine incorporation into soleus, EDL and heart amounted to 6·30, 5·20 and 5·84 nmol/g per min (*n* 9), whereas in animals Mg-depleted for 16 d the corresponding values were 5·66, 4·18 and 4·49 nmol/g per min (*n* 9) ($P < 0·05$, $P < 0·05$, $P < 0·02$ respectively). The incorporation of [^{3}H]phenylalanine in the control group amounted to 2·86, 2·75 and 2·41 nmol/g per min (*n* 8) in soleus, EDL and heart respectively. The values in animals Mg-depleted for 17 d were 2·16, 1·88 and 1·96 nmol/g per min (*n* 7) respectively ($P < 0·002$, $P < 0·001$, $P < 0·05$ respectively).

After 17 d on Mg-deficient fodder the K content of soleus muscle was 79·0 (SE 2·4) μmol/g wet weight (*n* 6) and 83·0 (SE 1·59) μmol/g wet weight (*n* 8) in the control group (not significant).

Zn depletion

Fig. 2 shows the effects of Zn-deficient fodder on growth in the age-range 4–11 weeks. Rats fed on the Zn-deficient diet showed an early reduction in growth rate. Following 7 d on Zn-deficient fodder the body-weight was significantly reduced, and after 17 d the weight gain amounted to only 26% of that of the controls. The relative reduction in wet weight of soleus and EDL muscles was almost the same as that of the body-weight (Table 3). A pair-feeding experiment showed that this growth retardation could not be attributed to reduced intake of fodder (see p. 499).

When Zn-depleted animals were repleted, they showed an immediate increase in body-weight and in growth rate. During the following weeks, however, the growth rate declined,

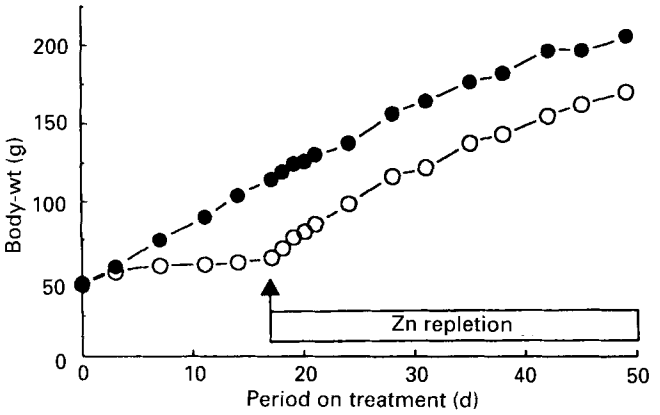

Fig. 2. Effect of zinc depletion and Zn repletion on body-weight. Groups of 4-week-old rats were maintained on Zn-deficient fodder (27 μmol/kg) or on the same fodder supplemented with zinc chloride to a content of 500 μmol/kg (for details of composition, see p. 494). After 17 d, the rats on Zn-deficient fodder were Zn repleted by changing to the Zn-supplemented fodder. Each point represents the mean of five Zn-depleted–repleted (○——○) or six control (●——●) animals. The standard error does not exceed the size of the symbols. The difference between the body-weight of the controls and that of the Zn-depleted–repleted rats was significant ($P < 0{\cdot}002$ or less) at all points except on days 0 and 3.

and the Zn-depleted–repleted animals did not reach the same body-weight as the controls during the course of this experiment. After 42 d of Zn repletion the weight difference between the Zn-depleted–repleted animals and the controls amounted to 32 g (values not shown on Fig. 2).

Since the administration of Zn-deficient fodder leads to reduced energy intake and ensuing impairment of growth and protein synthesis (Giugliano & Millward, 1984; Southon *et al.* 1985), the role of energy and Zn supply was analysed in a pair-feeding experiment. Table 3 shows that following 15 d on Zn-deficient fodder, the Zn-deficient animals had a weight gain amounting to only 15% of that seen in the pair-fed control animals. It should be noted that in the pair-fed controls weight gain was the same as that in the *ad lib.*-fed rats. In soleus muscle, the Zn content was the same in all three groups, but in EDL there was a small, insignificant increase in the Zn content of 7%. Only in the heart was there a small (15%) but significant loss of Zn in the Zn-depleted animals. It should be noted that in soleus the Zn content is three to four times larger than that in the heart and EDL. In the pair-fed control group growth or [^{3}H]leucine incorporation showed no reduction compared with the *ad lib.*-fed group. In contrast, in the Zn-depleted group, the [^{3}H]leucine incorporation into soleus, EDL and heart was reduced by 59, 57 and 64% respectively, compared with the pair-fed controls (Table 3).

The incorporation of [^{3}H]leucine into muscle protein was evaluated after 17 d of Zn depletion and in a group of animals which had been fed on the Zn-deficient fodder for 17 d and then had received the Zn-supplemented fodder for 5 d. Table 4 shows that in the three skeletal muscles studied (soleus, EDL and diaphragm) Zn deficiency was associated with a decrease in the incorporation of [^{3}H]leucine amounting to 35, 45 and 41% respectively. Also, in the heart, a pronounced reduction (45%) was seen. After 5 d of Zn repletion the [^{3}H]leucine incorporation had increased twofold, reaching values significantly above control levels in soleus, EDL and diaphragm muscle, but not in the heart. After 17 d on Zn-deficient fodder the K content of soleus muscle was 86·3 (SE 2·2) μmol/g wet weight (n 5) and 89·6 (SE 1·4) μmol/g wet weight (n 6) in the controls (not significant).

Table 3. *Effect of zinc depletion and pair-feeding on body and muscle weight, Zn content and [^{3}H]leucine incorporation in skeletal muscle and heart ventricle of rats*

(Mean values with their standard errors; no. of animals in parentheses)

Treatment*...	*Ad lib.*-fed controls		Pair-fed controls		Statistical significance of difference: *P*	Zn-depleted		Statistical significance of difference from pair-fed controls: *P*
	Mean	SE	Mean	SE		Mean	SE	
Body-wt (g)								
Day 0	48·5	1·0 (6)	48·7	1·2 (6)	NS	48·0	0·9 (5)	NS
Day 15	117·5	3·4 (6)	114·0	4·5 (6)	NS	58·0	1·1 (5)	< 0·001
Muscle wet wt on day 15 (mg)								
Soleus	46·2	2·8 (6)	45·1	1·8 (6)	NS	23·4	1·3 (5)	< 0·001
EDL	39·3	3·0 (6)	37·1	1·9 (6)	NS	23·9	1·8 (5)	< 0·001
Muscle Zn content on day 15 (μmol/g wet wt)								
Soleus	0·815	0·023 (6)	0·790	0·014 (6)	NS	0·824	0·017 (5)	NS
EDL	0·220	0·003 (6)	0·225	0·003 (6)	NS	0·240	0·014 (5)	NS
Heart	0·307	0·011 (6)	0·295	0·003 (6)	NS	0·252	0·010 (5)	< 0·002
[^{3}H]leucine incorporation on day 15 (cpm/g wet wt)								
Soleus	12630	524 (6)	12660	619 (6)	NS	5200	649 (4)	< 0·001
EDL	9249	262 (6)	8932	476 (6)	NS	3850	511 (4)	< 0·001
Diaphragm	10375	733 (6)	11066	486 (6)	NS	3991	338 (4)	< 0·001
Heart	9349	765 (6)	10889	320 (6)	NS	4448	343 (4)	< 0·001

EDL, extensor digitorum longus; cpm, counts/min; NS, not significant.
* For details, see pp. 494–495.

Table 4. *Effect of zinc depletion and subsequent repletion on [^{3}H]leucine incorporation (counts/min per g wet wt) into skeletal muscle and heart ventricle of rats*

(Mean values with their standard errors; no. of animals in parentheses)

Treatment*...	Controls (17 d) (*n* 8)			Zn-depleted (17 d) (*n* 7)		Controls (22 d) (*n* 8)			Zn-depleted (17 d) and Zn-repleted (5 d) (*n* 8)	
	Mean	SE		Mean	SE	Mean	SE		Mean	SE
Soleus	9868	522	$P < 0·001$	6405	566	9193	562	$P < 0·001$	12272	320
EDL	7184	630	$P < 0·001$	3959	618	8494	530	$P < 0·05$	9902	392
Diaphragm	9080	573	$P < 0·001$	5357	491	10169	470	$P < 0·05$	12144	776
Heart	11343	470	$P < 0·001$	6199	492	12235	1182	NS	11955	1115

EDL, extensor digitorum longus; NS, not significant.
* For details, see pp. 494–495.

DISCUSSION

The present study was performed with the purpose of comparing growth and in vivo protein synthesis during Mg or Zn deficiency states with tissue levels of Mg and Zn. The results indicate that reduced supplies of each of these elements produce inhibition of growth and in vivo protein synthesis in spite of small or no reductions of the tissue concentrations. In contrast, the serum concentrations showed a marked and early reduction following the administration of Mg- or Zn-deficient fodder (see Dørup *et al.* 1991).

Growth retardation

As in earlier studies, insufficient supplies of Mg or Zn both resulted in retarded growth rates, but growth inhibition during Mg deficiency seems slower in onset and is much less pronounced than that seen during Zn deficiency.

Since inadequate supplies of both Mg and Zn may influence food intake, it is important to perform valid pair-feeding experiments. Frequently, pair-feeding studies have been performed by offering the control group the same absolute amount of fodder as consumed in the deficient group on the previous day. It is our experience that in young animals this treatment, when continued for more than a few days, leads to severe discomfort and even death in the pair-fed group of animals because they are offered an inadequate amount of energy for their body size.

To prevent the semi-starvation of the pair-fed animals we chose instead to feed them the same relative amount of fodder, i.e. the same amount, on a body-weight basis, as that consumed in the group which received restricted Mg or Zn supplies (relative pair-feeding). This would make the pair-fed and the deficient animals comparable in relation to energy supply, and the only variable factor would be the supply of the element in question. For further discussion of the problems of pair-feeding see Dørup *et al.* (1991).

The present pair-feeding experiments confirm that both Mg deficiency and Zn deficiency *per se* have a restrictive effect on growth independent of the reduction in food intake. In addition, evidence is presented that this effect may be even larger than earlier suspected.

Tissue concentrations

The growth retardation was accompanied by pronounced reductions in serum values of Mg or Zn (see Dørup *et al.* 1991), but neither during Mg deficiency nor during Zn deficiency did the tissue concentrations of Mg or Zn show noteworthy alterations. In accordance with other studies (Cassens *et al.* 1967; O'Leary *et al.* 1979) we found that the Zn content of the red soleus muscle is about four times higher than that of the white EDL muscle. Our values for muscle Mg and Zn are in good agreement with those reported by others (Williams & Mills, 1970; Hunt, 1971; Giugliano & Millward, 1984).

Some studies on tissue Mg content during Mg deficiency have shown a small reduction in skeletal muscle Mg (Martindale & Heaton, 1964) whereas others could not detect any change (Hunt, 1971). In our study, even 2 weeks of Mg depletion did not significantly reduce the Mg content of skeletal muscle or the heart. Likewise, 2 weeks of Zn depletion caused no detectable reduction of the Zn content of skeletal muscle and only a small decrease in the Zn content of the heart. This is in accordance with earlier studies (Jackson *et al.* 1982; Giugliano & Millward, 1984). More importantly, the impairment of growth and protein synthesis was evident before any reduction in tissue concentrations could be detected. Following 2 weeks on Mg-deficient or Zn-deficient fodder the loss of K from the soleus muscle was modest and not significant (4·8 and 3·7% respectively).

In summary, very small changes in the skeletal muscle contents of Mg or Zn were seen and apparently the tissue concentrations were of minor importance for the ability to

synthesize protein. In contrast, the pronounced serum changes could be of importance in eliciting inhibition mediated by a regulatory (endocrine?) system.

[^3H]*leucine incorporation into muscle protein*

Both Mg deficiency and Zn deficiency were associated with reduced incorporation of [^{3}H]leucine into muscle protein. Like the growth retardation, this inhibition was more pronounced during Zn deficiency than during Mg deficiency.

Previous studies have shown that, in cell-free systems as well as in intact cells, Mg is necessary for optimum protein synthesis and that the protein synthesis rate varies in proportion to the intracellular Mg concentration (Schreier & Staehelin, 1973; Teresaki & Rubin 1985). George & Heaton (1978) measured incorporation of [^{3}H]leucine into protein in liver slices from Mg-deficient rats and found a 29% reduction, but did not report if there was a cellular Mg deficit. It should be noted that, in the study on intact cultured cells, a 43% decrease in [^{3}H]leucine incorporation was only seen when cellular Mg had dropped by 33% (Teresaki & Rubin, 1985). Schwartz *et al.* (1970) found that the in vivo incorporation of [^{14}C]valine into serum albumin was significantly reduced in livers from Mg-deficient rats. The same study showed a significant reduction in Mg content in the liver. To our knowledge, no earlier studies have evaluated the effects of Mg deficiency on muscle protein synthesis in an intact organism. Our results indicate that the reduced rates of growth and muscle protein synthesis cannot be accounted for by reduced total tissue Mg content. However, it cannot be excluded that, in the Mg-deficient rats, the free cytosolic Mg^{2+} was reduced. Similarly, differences in the intracellular concentrations of amino acids cannot be excluded. Measurements of the serum concentrations, however, indicate that Mg deficiency caused only minor changes in the extracellular pool of precursors for protein synthesis.

Differences in mean specific activity and mean concentration of the injected amino acid during the experiment could not explain the reduced incorporation of amino acid. The rate of [^{3}H]leucine incorporation into soleus muscle protein amounted to 6·30 nmol/g per min at a mean serum leucine concentration of 1·27 mmol/l. There seems to be a satisfactory agreement with our earlier in vivo and in vitro values. The value in our earlier in vivo experiments was 7·95 nmol/g per min, calculated on the basis of the injected specific activity and assuming a constant specific activity during the course of the experiment (Dørup & Clausen, 1989). In our in vitro experiments, performed at 30° and using a lower leucine concentration (0·5 mmol/l), the calculated value was 2·62 nmol/g per min. (Dørup & Clausen, 1989). The rate of phenylalanine incorporation into soleus muscle protein was 2·86 nmol/g per min. For both amino acids the calculated rates of incorporation of labelled amino acid were significantly reduced in the Mg-deficient rats.

The influence of Zn deficiency on protein synthesis in vivo and in vitro have been more intensively studied, and the general concept is that protein synthesis may be depressed in Zn-deficient rats (Hicks & Wallwork, 1987). Our study confirms the recent study of Giugliano & Millward (1987) who found a reduction in protein synthesis and a faster protein degradation in muscle tissue from Zn-deficient rats. Since in the latter study 'absolute pair-feeding' was used, the difficulty of separating the effect of Zn deficiency *per se* from that of the reduction in food intake would tend to underestimate the effect of Zn deficiency *per se*. The most important implications from our study are: (1) that Zn-deficiency *per se* has a restrictive effect on protein synthesis independent of the reduction in food intake, and that this effect may be even larger than earlier suspected; (2), that the effect on growth and protein synthesis is apparent without any detectable changes in the Zn contents of those tissues where protein synthesis takes place.

The effects of Mg and Zn deficiency on growth and protein synthesis have several

features in common. The reduction in growth and protein synthesis seen in Mg and Zn deficiency cannot readily be related to the loss of these elements from muscle tissue, nor to the loss of K. This suggests that the inhibition of growth and protein synthesis is not due to any local cellular deficiency, but rather should be seen as a response to a general deficiency. The most marked evidence of deficiency is seen in the serum levels. The role of serum contents and endocrine factors, especially IGF-1 will be further analysed in the following article (Dørup *et al.* 1991).

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